Summary

Lung cancer is the leading cause of cancer deaths worldwide for both men and women. Over 1 million people die each year from different types of lung cancer. Non-small cell lung cancer (NSCLC) accounts for approximately 80% of all lung cancer cases. The most common histological subtypes of NSCLC are adenocarcinoma (ADC), squamous target carcinoma (SCC) and large cell carcinoma (LCC). Among them, the ADC and SCC subtypes account for approximately 85% of all cases. Significant efforts have been made over the last decade to identify and validate new candidates for protein and small molecule biomarkers.

Omics research is an important part of the research on the discovery of potential protein biomarkers and metabolites, serving not only to define the subtype of NSCLC but also to detect and monitor the development of this tumor, especially at an early stage of cancer. Among the modern analytical tools available in the diagnosis of cancer diseases, techniques based on mass spectrometry, such as proteomics and metabolomics, give the greatest hope. Proteomics is a large-scale analysis of proteome protein expression. A proteomics is a set of proteins produced by an organism or found in a given biological system. On the other hand, metabolomics is a large-scale study of as many small biological molecules as possible, such as amino acids, organic acids, fatty acids, lipids, acylcarnitines and others. It is assumed the quantitative measurement of changes in the level of small molecules (metabolites) in the body are caused by physiological changes, pathological factors or genetic modifications.

The aim of the study was to analyze data obtained from large-scale proteomic and metabolomic analyzes that may indicate significant differences in the profiles of individual NSCLC subtypes, which may facilitate the interpretation of the obtained results and broaden the knowledge about the biology of this cancer. Moreover, large-scale omics data may indicate new physiological and biological aspects of cancer, thanks to which in the future it will be possible to better characterize the NSCLC subtypes, which will facilitate their diagnosis, as well as the differentiation of subtypes especially at an early stage of advancement.

The study group consisted of 99 patients with non-small cell lung cancer (72 men and 27 women) with a mean age of 64.5 ± 8.5 years and a predetermined histopathological subtype of NSCLC. Metabolomic analyzes were performed on samples from all patients. On the other hand, a subgroup of 45 patients (31 men and 14 women) was selected from the group of 99 patients for proteomic analyzes.

Proteomic analyzes were performed on the isolated protein, which was subjected to proteolytic digestion using the iFASP method. The obtained peptides were labeled with TMT isobaric tags, followed by the fractionation procedure. Peptide fractions were separated by nano-flow liquid chromatography and analyzed with an Orbitrap mass spectrometer. Protein identification was performed on the basis of FASTA sequence libraries using PEAKS X pro software. The analysis of biochemical pathways and possible protein interactions within individual comparisons was performed using Ingenuity Pathway Analysis (IPA) software. On the other hand, metabolites were extracted from tissue samples with methanol and then subjected to chromatographic separation by two methods (reverse phase and liquid hydrophilic interactions) and detection using a QTOF mass detector. The metabolites were identified on the basis of the obtained characteristic fragmentation spectra and compared with the spectra available in the databases. Pathway analysis was performed with IPA and MetaboAnalyst 4.5.

Early stage proteomic analyses indicated a potential activation of the LXR, GP6 and CDK5 pathways compared to the SCC and ADC subtype. In contrast to the ADC and LCC subtypes (early stage), it is characterized by a potential activation of the unfolded protein response pathway, glycolysis, gluconeogenesis and activation of sirtuin proteins in the ADC subtype. Metabolomic analysis comparing early-stage SCC and ADC showed that the metabolites creatinine, creatinine, xanthine and dihydrothymine are elevated in the SCC, while the fatty acid, carnitine, glycerophospholipids, lysoglycerophospholipids, amines, amino acids or amides are elevated in ADC. The group of glycerophospho- (N-acyl) -ethanolamine (GP-NAE) differentiating the early stage of SCC from ADC is also noteworthy. Metabolites differentiating the early stage of SCC advancement from ADC participate in many biochemical processes such as: Krebs cycle, urea cycle, respiratory chain and lipid degradation. The advanced stage is characterized by a potential activation of the LXR, GP6 and EIF2 pathways and an increase in the expression of proteins responsible for the remodeling of epithelial junctions in the SCC subtype with respect to the ADC. Comparing the ADC subtype to the advanced stage LCC indicates a potential activation of the LXR pathway and an increase in the expression of proteins responsible for the development of the inflammatory response and the acute phase reaction in the ADC subtype. In contrast, the potential activity of the glycolysis pathway in the ADC subtype was inhibited relative to LCC. The advanced stage LCC subtype is characterized by an increase in the sirtuin signaling pathway activity and an inhibition of the GP6 pathway and unfolded protein response pathway activity relative to the SCC subtype. Advanced-stage differentiating metabolites of the three subtypes are characterized by changes

in the levels of carnitines, amino acids, lipids and fatty acids. The largest differences in the level of metabolites are observed between the SCC subtype versus LCC and the ADC subtype versus LCC, where in each comparison the LCC subtype was characterized by a reduced level of metabolites. On the other hand, by comparing the individual NSCLC subtypes with the control tissue, we are debating an increase in the activity of potential biochemical pathways along with the increase in the stage of tumor advancement.

The obtained results indicate disturbances in the processes responsible for lipid and carbohydrate metabolism, as well as responses to unfolded proteins, which proves high oxidative stress taking place in the endoplasmatic reticulum. The analyses also indicate the dysfunction of the processes responsible for the process of translation and transcription, as well as glycolysis and gluconeogenesis in the early stage of NSCLC. However, in the advanced stage of NSCLC, the results indicate an increase in the activity of the processes occurring in the early stage of cancer advancement. The advancement of the stage is also characterized by an increase in the inflammatory response and acute phase response, as well as a response to remodeling of adherent epithelial junctions.